THE ROLE OF THE PFEIFFER BACILLUS IN THE RECENT EPIDEMIC OF INFLUENZA

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Since the recent visitation of influenza, which has been pandemic throughout the greater part of the civilized world, much has been written relative to its etiology and in particular to the part played by the influenza bacillus. While it is universally admitted that the pandemic disease of 1918-19 has not differed in any essential pathologic or clinical feature from that of the pandemic of 1890-92 when the cause was regarded as definitely established by Pfeiffer,[1] there is now considerable doubt, apparently, as to the etiologic relationship between clinical influenza and the Pfeiffer bacillus. Probably the main incentive for questioning the causal rôle of the Pfeiffer bacillus in the recent pandemic has been the severity and almost overwhelming clinical aspect of the disease prevalent in the infection. One was inclined to regard the picture as ascribable to some very virulent micro-organism or virus out of all accord in intensity of infection with the category of sporadic clinical entities promiscuously termed influenza, grip, and the like, usually noted in normal times. It must be recalled, however, that extraordinary circumstances existed in the massing of man power which formed the most desirable condition for intensifying the virulence of pathogenic microbes. Such an exaltation of the invasive power would explain the malignant character of B. influenzae when extending from military confines to adjacent municipalities. It must be remembered that the loss in virulence of greatly attenuated pathogenic species, long in stock as laboratory cultures, can be regenerated or exalted to a marked degree by animal passage. Certainly, in the conditions existing in military concentrations, the most desirable circumstances for repeated human passage were present.

In brief, there are three views extant today regarding the relationship of B. influenzae to the epidemic disease: (1) The acceptance of

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[1] Ztschr. f. Hyg. u. Infectionskrankh., 1893, 13, p. 357.

Pfeiffer's work and that of numerous confirmations by other reliable investigators;[2] (2) the view that the influenza bacillus plays only the rôle of a secondary invader, and that the disease is primarily caused by a filterable virus;[3] and a third view, that the Pfeiffer bacillus, together with other pathogenic micro-organisms of the respiratory tract flora, produces conjointly the syndrome called influenza.[4] Since the medical press has contained so many conflicting views concerning the etiology of epidemic influenza and the significance of various bacteria in the respiratory tract (the main dispute being over the part played by the Pfeiffer bacillus), we believe that our contribution to the study of the etiology will not be amiss at this time.

Our study is based on numerous cases of influenza in various hospitals in New Orleans during the primary and secondary outbreaks of the infection. Among the number are included 75 Porto Rican sailors ill with the disease, who were selected for study especially because they offered unusual opportunities in that they were all from the same source and clinically presented the same infection. Furthermore, the number was sufficient to form a basis for proper deductions from the results obtained. At the time of admission, approximately one-third of the number were apparently in the early stages of the disease, stating they had been stricken the day previous to their arrival in port, while others gave a history of being sick for a longer period. Several of these patients presented a well developed pneumonia. While under observation 17 of the 75 died and necropsies were made. Fifty-eight of the cases recovered after a period of from 2 to 6 weeks, and were discharged well.

The work consisted in (1) making cultures of the sputum, blood and tissues, especially from the lungs of those on whom necropsies were held; (2) gross and microscopic observations of the lesions found postmortem; (3) repeated blood examinations during the course of infection; and (4) serologic studies. In conjunction with the work approximately 5,000 persons were vaccinated with the protein of

[2] Weichselbaum, A.: Wien. klin. Wchnschr., 1892, 32, p. 459. Huber, cited in Kolle und Wassermann: Handbuch der Pathogenen micro-organismen, 1903, 3, p. 359. Baumler, cited in Kolle und Wassermann: Handbuch der Pathogenen micro-organismen, 1903, 3, p. 359. Robertson, W. F.: Brit. Med. Jour., 1918, 2, p. 680. Opie, Eugene L.; Freeman, A. W.; Blake, F. G., Small, J. C., and Rivers, T. M.: The Jour. Am. Med. Assn., 1919, 72, p. 556. Spooner, L. H.; Scott, J. M., and Heath, E. H., Jr.: Jour. Am. Med. Assn., 1919, 72, p. 155.

[3] Nicolle, Charles, and Lebailly, C.: Compt. rend. Acad. d. sc., 1918, 167, p. 607. Dugarrie de la Riviere, R.: Compt. rend. Acad. d. sc., 1918, 167, p. 606. MacCallum, W. G.: Jour. Am. Med. Assn., 1919, 72, p. 720. Gibson, H. G.; Bowman, F. B., and Connor, J. I.: Brit. Med. Jour., 1918, 2, p. 645.

[4] Lord, F. J.: Scott, A. C., and Nye, R. N.: Jour. Am. Med. Assn., 1919, 72, p. 188. Whittingham, H. E., and Sims, Carrie: Lancet, 1918, 2, p. 865.

killed influenza cultures and as the results have some bearing on the etiologic relationship of B. influenzae, certain aspects will be discussed.

BACTERIOLOGY

The sputum and blood were collected from all the cases and within two hours were plated (surface seeded) on complement free, human blood agar, 0.6% acid. Smear preparations from the specimens of sputum were also made as a tinctorial and morphologic index to the number of bacterial varieties in colonies on the plates. In this connection, however, it may be said that little dependence could be placed on bacterial morphology in the smears of the sputum as an aid in the identification of subsequent colony species because relatively small amounts are microscopically examined as compared to that planted, and furthermore morphologic features in vivo are not always repeated in vitro. The identity of the Pfeiffer bacillus was established by cultural characteristics and the behavior of the isolated culture toward known immune serum, employing for this purpose the agglutination and complement fixation tests.

The sputum from the 75 Porto Ricans yielded in every instance a bacillus corresponding in all essential characteristics to the micro-organism described by Pfeiffer. As a general rule, however, it was not the predominating colony on the plates, not even in instances of early infection. B. influenzae colonies were on the average more numerous on the plates from the cases of early infection than from those more advanced. In the latter we invariably encountered greater numbers of pneumococci and streptococci, which fact we attributed to the advent of secondary invasion in these cases.

The influenza colonies were slow in appearing on the plates, often not visible even with a hand lens under 48 hours incubation, while other bacterial species were well advanced or had by this time completed their colony growth. The slowness with which the influenza colony appears in its initial growth is to be anticipated in all work on its isolation from contaminated human material, and especially sputum, which usually contains a rich saprophytic flora. The mature influenza colony at best is often relatively minute as compared with those of other associated species, and in consequence may be overlooked unless careful attention is paid to this point. Not infrequently, when streaks were made in the seeding of plates, we noted merely a roughened tract or loss of surface gloss which on examination with a hand lens revealed numerous closely set, pin-point, transparent colonies of B. influenzae. We regard inattention to these points as probably accountable for the numerous reports of failure to isolate B. influenzae from the epidemic cases. Even where numbers of B. influenzae are present they may be inhibited in their development by the alterations in the medium brought about by the growth of other species. The antagonism in this respect is especially marked in the instance of the pneumococcus and streptococcus. On the other hand, we found the presence of the staphylococcus advantageous since it favored the growth of the influenza bacillus under artificial conditions.

We did not attempt the systematic isolation of the Pfeiffer bacillus from socalled normal throats or from the nose and mouth material of those suffering with other respiratory diseases; however, in those examined we occasionally found the Pfeiffer organism to which we attributed no significance as influenza was widespread throughout the community, and therefore its presence

was to be expected in many upper respiratory tracts, normal and otherwise.[5] The incidence in normal throats and throats of recovered persons, however is interesting since it explains the survival through human carriers over the interval between epidemic outbreaks and the sporadic occurrence of the disease. The demonstrated occurrence of the meningococcus under similar circumstances[6] forms an analogue in an accepted causative factor which readily permits of comparison. The finding of B. influenzae, therefore, in apparently normal throats months after the passing of an epidemic would not be evidence of any moment against its causal rôle in the disease known as influenza.

Blood cultures were negative for B. influenzae in all the cases examined; it was, however, recovered from the heart blood in three fatal pneumonias. In one it was present alone and in the two others associated with the hemolytic streptococci. It would seem that only occasionally, and then under conditions not entirely understood, the Pfeiffer bacillus gives rise to a septicemia.

The Pfeiffer bacillus was recovered from the pneumonic lungs at necropsy in 16 of 17 cases, but in none was it present in pure culture or even as the predominating species. We lay no stress on this point because the number of colonies developing in vitro is never an index to the number in the lesion as many at the time of plating may not have been viable, and others, highly parasitic, may not have been able to adjust themselves to the new artificial environment. The colonies on the plates were intermingled with those of the streptococcus, pneumococcus and staphylococcus, and occasionally with other undetermined bacteria species. The relative proportion of B. influenzae and those of the pneumo-streptococcal varieties were, as a rule, like that observed in the sputum of late cases of the disease. In certain of the cases wherein numerous small abscesses were scattered through the pneumonic lung, the predominating organism recovered was the staphylococcus aureus.

PATHOLOGIC ANATOMY

Since we are concerned especially with the relationship of B. influenzae to the respiratory lesion, only the tissue reaction of this system, and particularly that of the lungs, will be considered. The primary lesion of the respiratory tract is an intense acute nonsuppurative catarrhal inflammation of the mucosa and submucosa, involving the bronchial tree and often its finer terminals. These structures are tumefied and of a mottled dusky red color and the surface is moist and finely granular in appearance. The essential host reaction produced by B. influenzae corresponds in general to the gross picture occasioned locally in the tissues of animals following the injection of diphtheria toxin. This picture is later transformed to the ordinary gross lesion characteristic of secondary pyogenic invasion of the tissues.

Occasionally we encountered a rapidly fatal case in which the lung showed no gross evidence of consolidation but extensive areas in which the parenchyma was filled with a bloody fluid. There were usually present localized areas showing emphysema and dilatation of the bronchioles. Around the latter were grayish concentric patches which microscopically revealed a serofibrinous exudate. If the causal agent of influenza per se produces a distinct type of pneumonitis, this picture might be so regarded. On the other hand if this lesion is not to be attributed to B. influenzae it becomes necessary to regard it as the preneutrophilic or serum stage of exudation that represents the early

[5] Pritchett, Ida, W., and Stillman, E. H.: Jour. Exper. Med., 1919, 29, p. 259.

[6] Flexner, Simon: Mode of Infection, Means of Prevention and Specific Treatment of Epidemic Meningitis, 1917.

phenomena occasioned by secondary pyogenic invasion. The usual pneumonia seen at necropsy in our cases and regarded as produced by secondary infections, was definitely lobular in type, though in a few the extensiveness of the consolidation made it appear lobar on gross examination; however, on microscopic study its lobular character was established. As a rule, the pneumonitis with definite areas of consolidation presented nothing unusual either in the gross or microscopic appearance which would justify one in regarding the lesion as different from the lobular pneumonia caused by a variety of pyogenic micro-organisms. Our findings correspond in general to those of Wolbach,[7] who describes two predominating types of pneumonia regarding them as different stages of the same process. However, he considers both types as caused by B. influenzae.

Multiple miliary abscesses occurred in many of the lungs seen by us at necropsy and not infrequently there was extensive hemorrhage in the lobes involved and myriads of petechiae on the pleura, which reminded us of the lung picture in bubonic plague. Our bacteriologic findings would indicate in these instances that secondary invaders are responsible for the ordinary pneumonic complication present and not the influenza bacillus per se.

LEUKOCYTIC REACTION

Our observations on clinical influenza revealed in the early stages of the disease little, if any, response on the part of the circulatory leukocytes; in fact, at this period there was a definite leukopenia. When pneumonic complications occurred there was invariably a definite rise of the normal count; this increase is to be attributed to secondary infection. As there is a constant leukopenia early in the disease, and as leukocytosis does not occur until the pneumonia has been superimposed, it would appear that the latter was occasioned by a secondary invader and not by the primary excitant of the infection. Leukocytosis may exist without pneumonic involvement during the course of the disease wherein secondary invasion of the upper respiratory tract has occurred. The total leukocytic count remained below 10,000 in the uncomplicated cases while in those presenting secondary lesions the count reached as high as 18,000. In the very early cases of influenza the counts were usually below 6,000. In the instances where there were complications with high leukocytic counts, the increase was due to the polymorphonuclear neutrophil.

SERUM REACTIONS

The blood from about 200 influenza patients representing all stages and periods of the disease, was tested for agglutination with the Pfeiffer bacillus. Varying dilutions of the patient's serum were employed, ranging from 1:20 to 1:100, and the final readings were made 24 hours after the mixtures were set up. The readings after from 18 to 24 hours have been found preferable because the agglutination reaction is slow, and readings made too early may therefore be misleading. The blood of patients, with few exceptions, gave very definite agglutination of B. influenzae, many reacting in dilution 1:80. Not infrequently the reaction was obtained as early as the third day of the disease. Normal blood used for control rarely reacted to the Pfeiffer bacillus and in the positive instances not in a serum dilution above 1:20.

The complement fixation test for the determination of specific lysin was also carried out on the series of cases. Specific lytic substance for B. influenzae antigen was not detected as a rule as early as the agglutinin; however,

[7] Wolbach, S. B.: Bull. Johns Hopkins Hosp., 1919, 30, p. 104.

in the later stages of the disease, particularly in those recovering, the reaction was fairly constant.

In our opinion the specific serum reaction obtained in influenza patients is deserving of more than passing interest. It has, perhaps, as great a significance from the standpoint of etiology for the Pfeiffer bacillus as any other postulate.

B. INFLUENZAE PROTEIN SPECIFICITY

The definite antigenic property of the Pfeiffer organism, as indicated by the serum behavior of patients and determined by animal experiments, led us to attempt the protection of the human against the infection with the specific protein of the freshly killed culture. Approximately 5,000 persons were vaccinated, each receiving three separate subcutaneous injections of 1,000,000,000 organisms at intervals of 3 days. The detailed report of the results obtained with vaccine will appear in a separate paper. Our intention here is to give only the points of interest which have a bearing in the establishment of a causal rôle for B. influenzae.

In the preparation of the specific protein several isolations of B. influenzae from recent cases of the disease and a culture from the Rockefeller Institute, which we have maintained as a stock culture for approximately five years, were employed. Wide variations in the immunizing power of the killed influenza culture depended to a large extent on the manner in which the protein was prepared. Heat as an agent for destroying the viability, altered in a greater or lesser degree the antigenic property. Likewise tricresol and other derivatives of the phenol group affected the immune-body production of killed B. influenzae protein. The maximum potency of the vaccine was obtained with chloroform as the bactericidal agent. This chemical apparently does not affect in the slightest degree the toxicity and because of its volatile property is quickly eliminated from the killed protein product.

The injection of the protein material gave a decided local and constitutional reaction in fully 90% of those vaccinated. In 30% the reaction was strikingly similar to that noted in influenza infection. A number of those inoculated were obliged to go to bed in from 6 to 8 hours after the administration of the first injection and to send for a physician who not infrequently pronounced the case influenza which subsequently proved to be only the severe constitutional reaction occasioned by the specific foreign protein. In general, it can be stated that the reaction ranged from a slight headache and mild pains over the body with a temperature of one-half to one degree F., lasting not more than 24 hours, to that of severe headache and neuralgic pains, ushered in by nausea, vomiting and chill and temperature of 101-102. It is noteworthy that in persons showing the more severe reactions the duration was shorter than in those in whom it was less severe.

In addition to the constitutional reaction there occurred a local transient inflammation at the site of inoculation. This ranged from a mild circumscribed erythema, 4-5 cm. in diameter to a markedly swollen and reddened skin involving the whole arm and greater part of the forearm. However, the intensity of the reaction was no index to the degree of constitutional effect from the vaccine. Often the constitutional reaction was associated with but little local inflammation at the inoculation site and conversely, the person developing a reddened and severely swollen arm often showed little fever and complained of no other indisposition.

The blood of the vaccinated without exception revealed the antigenic property of killed B. influenzae in the form of specific agglutinin in 36 hours after the first injection. This substance increased in the circulation to a significant degree after the second and third inoculations.

SUMMARY AND DISCUSSION

The work herein described had for its purpose the investigation of the specific pathogenicity of the organism known as B. influenzae. Based on a careful study of a representative series of influenza cases, we believe there is adequate proof to show that the Pfeiffer bacillus or a possible allied strain is the cause of the disease known as influenza which occurred in epidemic form in the early fall and winter of 1918. It would seem that sufficient postulates for the recognition of its etiology have been fulfilled.

In our opinion the influenza bacillus may be recovered from all cases of epidemic influenza. It was obtained from the sputum in every instance, and from the pneumonic lungs in 94 per cent. of the cases in which necropsies were held. Cultures from the heart blood yielded negative results in all except three of the fatal pneumonias. Here in addition to B. influenzae there was recovered the pneumococcus, streptococcus, staphylococcus and other undetermined species. In all cultures from the sputum and pneumonic lungs we encountered greater numbers of the pneumococcus and the streptococcus than we did B. influenzae. As the upper respiratory tract has normally a rich bacterial flora, it was not surprising to have some one or more of these organisms overshadow B. influenzae to such an extent that the isolation of the latter was accomplished with difficulty. It cannot be denied that the Pfeiffer bacillus is far more parasitic than any of the ordinary species found in the upper respiratory tract, which often accounts for the comparatively few colonies of B. influenzae that develop, or their failure to grow on special cultural media. Not infrequently attempts to isolate the Pfeiffer bacillus from the sputum of the late stage of the infection are unsuccessful because the organism is either there in too few numbers or is no longer viable, having been crowded out by the less parasitic and in consequence antagonistic bacteria of the normal flora. Again, the Pfeiffer bacillus in certain sputums may not be hardy enough to survive the change of environment produced by conditions of artificial cultivation, especially when transplanted with relatively large numbers of other less parasitic bacteria.

The finding of B. influenzae in the so-called normal nose and throat, and in various other lesions of the respiratory tract, which has been advanced by some[8] as evidence against its specific causal relation to

[8] Kinsella, R. A.: Jour. Am. Med. Assn., 1919, 72, p. 717. Lord, F. T.; Scott, A. C., and Nye, R. N.: Jour. Am. Med. Assn., 1919, 72, p. 188.

influenza, cannot from this standpoint be seriously considered. It has been definitely established that the bacillus may sojourn in the upper respiratory tract for months after recovery from the infection; and, furthermore, in the light of our knowledge of human carriers of other bacteria, such as B. diphtheriae, meningococcus, etc., it is entirely consistent with parasitism for persons not susceptible to harbor the parasite for a longer or shorter time.

In our opinion influenza begins primarily in the mucous membrane of some part of the respiratory tract and may spread rapidly from the original focus to the mucosa of the nasopharynx, larynx, trachea, bronchi and bronchioles. While the disease is essentially a toxemia, there occasionally occurs a bacteremia which would account for certain extrarespiratory lesions other than those not due purely to the specific toxin or secondary pyogenic invaders.

The microscopic changes indicate that the pneumonitis with definite consolidation is a superimposed lesion and not produced by the Pfeiffer bacillus per se. It is commonly the result of secondary pyogenic infection which is brought about by the injury to the tissues of the lower respiratory tract, occasioned by the influenza bacillus. While an acute inflammatory exudate is the common finding in the mucous membrane, we do not believe it represents the true lesion occasioned by B. influenzae but rather that produced by secondary invaders. The neutrophilic type of lesion in the respiratory tract is not surprising when we consider that normally in this situation there is a rich bacterial flora, members of which under a variety of conditions are capable of acting as secondary excitants. In consequence of the primary injury to the mucous membrane secondary infectious processes are common, and in particular those due to the pneumo-streptococcal group. These organisms are accepted as secondary invaders in other pathologic states of the air passageways; for example, in diphtheria and the acute exanthems, and therefore it is consistent to consider that they can play the same rôle in influenza.

The lesion in the respiratory tract of influenza in consequence of its site does not represent the true tissue reaction caused by any one pathogenic excitant, but the composite picture produced by a number of micro-organisms. Therefore, in describing the lesion one must not attempt to characterize it as pathognomonic of a particular entity. To form any idea of the specific host reaction to influenza infection one must study the changes in tissues that are removed from possible

source of confusion occasioned by coexisting micro-organisms. For example, pure influenza meningitis would afford a more desirable opportunity to observe the uncomplicated tissue reaction. Here it is plain that the essential change is lymphocytic and associated with proliferation.

Although the tests in animals with the living Pfeiffer bacillus have not been accepted as entirely satisfactory, certain results obtained by us in man with the injection of the chloroform killed influenzal cultures has definitely demonstrated its toxic qualities. During the epidemic we had occasion to vaccinate over 5,000 persons, and in fully 30 per cent. the constitutional reaction was strikingly similar to the early syndrome of the natural infection. We do not wish to infer that this reaction to influenzal protein is proof of the living organism's etiologic relationship; nevertheless, it is significant and therefore to be weighed in the consideration of the causal factor.

We regard the development of specific immune substances in the host during the course of the disease as of sufficient evidence to establish etiologic relationship. In practically all of our cases the agglutinin for the Pfeiffer bacillus was demonstrated and the lysin in some. The agglutinin was in evidence in the blood too early in the disease to be explained simply as the result of the activity of the Pfeiffer bacillus as a secondary invader. The specific agglutinin appeared often as early as the third day of the infection and rose steadily in amount as the disease progressed for those who made an uneventful recovery. In the rapidly fatal pneumonic cases the agglutinin was relatively low, and in several not detected. Controls usually gave no reaction for the Pfeiffer agglutinin in those who had not recently had influenza.

CONCLUSIONS

The micro-organism known as the bacillus of Pfeiffer may be isolated from the material of the respiratory tract lesion in all cases of epidemic influenza, and recovered only occasionally from persons not infected.

There is inadequate proof that the Pfeiffer bacillus is a member of the normal upper respiratory tract flora. That it may occur in normal individuals during epidemic times or persist for months in those who have recovered from the infection, is well recognized. These persons are the interim carriers of the bacillus and constitute an important means for transmission of the infectious agent.

During the course of the infection and for a variable time after recovery, the blood contains specific immune bodies for B. influenzae while those not infected are without these substances.

The subcutaneous inoculation of persons with influenza protein causes the production of specific immune bodies. The reaction occasioned in the inoculated person is definite evidence of its toxic property.

Secondary infections with one or more of the ordinary respiratory tract flora is common in epidemic influenza and is usually responsible for the occurrence of pneumonia.

The Pfeiffer bacillus is the primary cause of epidemic influenza for the reason that sufficient postulates in the recognition of its etiology can be fulfilled.